RISK EVALUATION OF FOOD AND ENVIRONMENTAL CONTAMINANTS

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"Risk assessment. Risk management. It has become fashionable to separate the consideration of risk into two parts. Risk assessment - the estimation of the association between exposure to a substance and the incidence of some disease - is presumably a job for the scientist. Risk management is the process of deciding what to do about the risk once determined. It calls upon many disciplines. When the risk affects human health or the environment, the job usually lands on the desk of a public policymaker" (David Durenberger).

PREAMBLE

As the popularity of the terms "risk assessment" and "risk management" has increased, varied interpretations of their meaning have proliferated. It is therefore important to give from the onset a general idea about the conceptual content of each term.

Risk assessment: at one extreme, the term is used to encompass all societal functions related to risk, from the identification that a risk exists to implementation of risk reduction measures. At the other extreme, the term has been limited to the methods used to quantitatively extrapolate human cancer risks from toxicological studies on animals. The term can be generally described as the process of determining the adverse consequences that may result from the use of a technology or some other action. The assessment of risk typically includes: (1) an estimate of the probability of the hazard occurring; (2) a determination of the types of hazard posed; and (3) an

estimate of the number of people or things likely to be exposed to the hazard and the number likely to suffer adverse consequences.

When defined in this manner, risk assessment can be used for several purposes: to establish priorities for further risk assessment or for research; to inform the public about risk; and, as part of the regulatory process, to decide what risk should be regulated and what the content of the regulations should be. A determination of what actions should be taken to control a risk moves the process from risk assessment to "risk management".

Risk management: it encompasses all activities in actually doing something about a risk. First comes a decision on whether any actions are necessary, and if so, what the nature of the actions should be. This decision must be based not only on measuring risk, but also on judging the accetability of that risk, a matter of personal and social value judgement.

In addition, risk management include implementing the actions decided upon and evaluating their effect.

In short, risk management decisions are always grounded in some sort of risk assessment, although this may be no more than a decision maker's assumption about the seriousness of the risk.

INTRODUCTION

Risk assessment is usually the firts step in risk management and procedures are directed to identifying adverse effects, establishing management criteria and setting legal exposure limits. Thus, the procedures are often legal administrative instruments and subject to national prerogatives and practices. However, there is wide international agreement on the toxicity tests which are used and the fundamentals of these are described. In all risk assessments, it is essential that the scientific basis - toxicologial and ecotoxicological -is maintained and not supplanted by purely administrative procedures.

It is of considerable importance the laboratory test data are reliable because they are central to risk assessment. While sound data will not necessarily result in a good risk assessment, due to the variables involved in the process, it is certain that bad, poor or inadequate data will never produce a good assessment.

When dealing with risk and hazard it is essential to define terms. They are often used synonymously or inconsistently to denote either possibility or probability. To achieve some uniformity of usage within the environmental health components of the World Health Organization these, and some related terms, are defined as:

Risk - A quantitative probability that a health effect will occur after a specific "amount" of a hazard has exposed an individual.

Hazard - A source of danger; a qualitative term expressing the potential that an environmental agent can harm health.

Risk Estimation - The quantification of dose-effect and dose-response relationship for a given environmental agent, showing the probability and nature of the health effects of exposure to the agent in a general scientific sense.

Hazard Identification - The identification of the environmental agent of concern, its adverse effects, target populations and conditions of exposure.

Exposure Assessment - The quantification of the amount of exposure to the ASSESSMENT hazard for an individual or a group.

Risk Characterization - The outcome of "hazard identification" and "risk estimation" applied to a specific use or occurrence of an environmental health hazard (e.g. a chemical compound). The risk characterization thus requires quantitative data on the human exposure in the specific situation. The end product is a quantitative statement about the proportion of affected individuals in a target population.

Risk Assessment - A combination of the four steps: *Hazard Identification - Risk Estimation - Exposure Assessment - Risk Characterization.*

Risk Evaluation - The comparison of calculated risks or public health impact of the exposure to the environmental agent with risks caused by other agents or societal factors together with the benefits associated with the agent. This may lead to a decision about "acceptable risk".

Exposed or non-Exposed - Qualitative terms defining the existence of or lack of EXPOSED hazard in the environment of individuals.

Exposure or Dose - Quantitative terms defining the amount of an environmental agent that has reached the individual (external dose) or has been absorbed into the individual (internal dose, absorbed dose).

PERCEPTION OF RISK

The existence of risks to health has been an integral part of life since evolution began, but recently, attitudes to, and acceptance of, risk have undergone mayor changes. In the evolutionary process, organisms have had to adapt and adjust to endogenous and exogenous toxic chemicals in order to survive. The human race has adapted through natural selection and physiological processes and also culturally by taboos and dietary patterns designed to avoid or minimize exposure. Conscious social adjustments now include the legal regulation of risky activities. Risks are no longer regarded as unavoidable.

A significant event in the public perception of chemical risk has been the rapid growth of environmental protection movements which consider chemicals as one of the major threats to human kind. Environmental concerns extend beyond the chemical pollution of air, land and water, to cover nuclear power plants, noise in the urban environment, industrial installations particularly of the chemical industry, and including the destruction of the ozone layer. Pesticide reccive particular attention, followed by environmentally persistent chemicals such as organochlorine compounds and environment threatening chemicals such as chlorofluorocarbons. The public concern for human and environmental health has been sharply focused by events, such as pollution incidents in Italy (Seveso, dioxin), Japan (Minamata Bay, mercury), England (London, smog), U.S.A. (Michigan), and Taiwan [polybrominated biphenyls (PBBs)] and chemical warehouse fires in Switzerland, Australia, and England which polluted waterways. These incidents have been among the driving forces for imposing controls on potentially toxic chemicals. The awareness of environmental threats was rapidly followed by a realization of the dangers of delayed human health effects, such as cancer or genetic diseases, due to exposure to toxic chemicals for long periods of time at low concentrations.

Chemical risk assessment is a crucial part of ensuring human and environmental health. In many countries risk assessments are made for all chemicals produced or imported and for industrial developments (industrial impact assessment) to ensure that there will be no adverse health effects.

COMPONENTS OF RISK ASSESSMENT

Risk assessment is not a new discovery. It has been at the core of the insurance business for centuries starting with risk assessment for shipwreck and cargo loss. These risk assessments were relatively straightforward because they were based directly on a large body of real data: well into the last century shipwrecks were common. Life insurance also developed on the basis of actuarial experience. The pace of development of sophisticated statistical risk assessment techniques for predicting failure rates and thus reliability for aircrafts and mechanical components. Risk assessment is widely applied in calculating insurance premiums.

The term risk assessment, as used in this paper, comprises the identification of possible underisable effects (hazard), the likelihood of occurrence and magnitude of these effects (estimation of risk), the quantitative assessment of exposure and, finally the risk characterization which is a quantitative statement about effects and the proportion of a population which will be affected. Risk assessment is a scientific process which precedes risk evaluation and risk management and control measures.

Hazard identification can be predictive or actual. If actual, it may follow observation of adverse effects on humans and other biota or the environment or the detection by environmental monitoring of toxic chemical pollution. Hazard identification has advanced rapidly because of the development of knowledge on the ways in which chemicals can adversely affect health. This has led, worldwide, to demand, that chemicals are tested thoroughly before production and sale. However, attention has focuses largely on "new" chemicals and little has been done about the systematic testing of the tens of thousand of chemicals already in use, unless there are major grounds for suspicion.

For "new" chemicals, notification schemes have been introduced by many countries. Notifications usually contain specified health, environmental and physical and chemical properties test data. In some countries the data requirements are specified in detail, while in others regulatory bodies must prescribe for each notifiable chemical the tests which are to be performed.

Prediction of lack of significant risk of a chemical do not remove the need for continued vigilance. For chemicals in use or present in the environment, even where safety seems assured, possible health hazards still need to be monitored. Epidemiology is one way of linking exposure to

chemicals with adverse effects but has not yet been fully exploited for studies on the effects of specific chemicals on general populations. There is also a role for clinical diagnosis and observation which have frequently raised the first suspicion that a chemical is the cause of a disease. In order to establish objectively the existence of a hazard, diagnosis in an individual needs to be followed by monitoring and epidemiological studies of population groups exposed to the same chemical(s).

EXPOSURE ASSESSMENT

Exposure assessment can be predictive or actual. At the pre-manufacture or pre-marketing stage it is predictive, although experience of chemically similar substances may sometimes be available. Predictive exposure assessment for chemicals which will disperse widely in the environment may be difficult. However, estimates can be made on the environmental behavior of chemically similar substances, from laboratory data on physical and chemical properties and the use of mathematical models of distribution in environmental compartments and chemical fate.

Exposure assessment at the post-marketing stages is often carried out for chemicals and formulations used in consumer products or for chemical food additives, residues or impurities. In these cases, the general environment is not a significant pathway of exposure. It is the quantities and use patterns of individuals and populations which are the main determinants of exposure. Exposure can be estimated by surveys of lifestyle and dietary patterns by, for example, "market basket" surveys, or measured by biological monitoring of human body tissues or fluids.

Exposure assessment for chemicals which have entered and dispersed in environmental compartments requires a combination of environmental monitoring and mathematical modelling. These assessments are resources intensive and need sophisticated analytical techniques and should only be performed after a thorough review of the problem. For significant chemical pollutants, both local and general exposure assessments may be necessary. In every case, it is essential to define the sources of the chemical pollutant, its locations and levels in the environment, and the time-course and variability of exposure. These data, combined with information on human and environmental health effects, contribute to the risk characterization and assessment of the overall health risk.

RISK CHARACTERIZATION

When a hazard has been identified, the nature, magnitude and probability of occurrence of adverse effects, the target populations and the exposure must be examined. The conditions of exposure should be defined in terms of concentrations, distributions in environmental compartments and trends and target groups. These are then linked to the dose-effect and dose-response (groups) toxicity relationship, in order to relate the predicted or actual exposure to the effects and their probability of occurrence. Estimation of dose-effect and dose-response relationships and the assessment of exposure are often complicated. Even for direct and relatively simple environment pathways, the estimation of measurement of exposure can present problems. The assessment of exposure resulting from intentional use or from accidental release of chemical presents different problems to those presented by chemical widely dispersed in the environment. For intentional use an assessment can be based on expected sites and levels of production, use patterns and quantities, complemented by data on physical and chemical properties. For widely dispersed chemicals the original sources and detailed environmental distribution may be difficult to establish. In summary the steps are:

Hazard Identification

The identification of the chemical and its inherent dangerous properties by knowledge of:

- Chemical and physical properties
- Toxicity
- Ecotoxicity
- Persistence in the environment
- Bioaccumulation
- Environmental mobility and fate

Risk Estimation

The quantification of dose-effect and dose-response, the type of adverse effects, their reversibility or irreversibility, threshold dose and no-adverse-effect level, using data from:

- laboratoy animals *in vivo*
- *in vitro* studies
- environmental biota under laboratory conditions
- field studies

Exposure Assessment

The quantification of exposure of targets or target systems, such as human populations, environmental species and/or ecosystems based on:

- environmental concentrations
- environmental distribution, pathways, fate
- receiving environments, compartments, target populations

Risk Characterization

The probability that a chemical would cause adverse effects as a result of specified production, use and emission into the environment, using data on:

- exposure (intensity, frequency and duration)
- routes of exposure
- toxicity and ecotoxicity

and concludes in a quantitative relationship between exposure and the proportion of a population likely to be affected.

A RISK ASSESSMENT PROCESS

Predictive risk assessment for humans is generally based on toxicity data from experimental animals. Human data would be the most relevant but with the exception of some dermal effects, testing of therapeutic agents, experimental information will not be available. However, where there has been human exposure to similar chemicals there may be clinical and epidemiological data which could help.

Although toxicity testing in animals is used for predicting health risks to humans, these tests do not provide absolute assurance that there is no significant human health risk. Acute toxicity testing in animals is useful in predicting similar effects and toxic doses for humans, but the situation is less clear for the effects of long-term exposure. Chronic toxicity tests in animals are used to establish "safe" levels for long-term human exposure. However the extrapolation of chronic laboratoy animal studies to humans depends on many factors, such as the test species, the routes of administration, the number and range of doses, the detailed design of the test procedure, and metabolic differences and similarities.

At the present time, it is not practicable because of cost and resource implications, to test chemicals for every possible toxic effect and testing strategies are used. For example, many countries use sequenced approaches with the steps related to the quantities produced, chemical use patterns, toxic and ecotoxic properties, the type of human exposure, and the exposed populations.

Because animal toxicity data are basis to risk assessment the tests commonly used merit a brief description. They fall into the following main categories:

- acute exposure
- local effects on the skin and eye
- allergic sensitization
- subchronic exposure
- chronic exposure
- effects on reproduction
- effects on the nervous system
- mutagenicity
- carcinogenicity
- toxicokinetics.

Internationally, there is good agreement on how these studies should be designed and carried out. Data produced for regulatory purposes in accordance with good laboratoy practice are ussually acceptable internationally.

While the use of mathematical modelling or quantitative structure-activity relationships as a part of risk assessment has become more common, risk assessment is still essentially a scientific judgmental process dependent on reliable data. In exercising judgement, it is essential to know what information a toxicity test can provide and what cannot. Speculation or specious extrapolation must be avoided.

ACUTE TOXICITY

The usual initial step is the acute toxicity test. It deals with the effects following immediately or soon after the administration of a single dose of a chemical or a series of doses over a short interval. The objective of this test is to examine the nature of acute toxic effects and not just the end-point of lethality. In an acute test, lethality is usually expressed as a median lethal dose, or LD-50, a statistically derived expression of a single dose of a material that can be expected to kill 50% of the exposed animals when administered by the oral or parenteral routes. A median lethal concentration, or LC-50 is used for lethality by inhalation or for fish in the aquatic medium and must be qualified by the duration of exposure to provide meaningful information. The LD-50 and LC-50 are widely used numbers, particularly for classification purposes, but they are crude measures of toxicity and have limited scientific usefulness. Unfortunately, they are widely and falsely assumed to represent the overall toxicity of a chemical.

Acute testing should establish the signs of acute poisoning, possibly indicate mechanisms, identify sensitive systems or organs and determine if the effects are reversible. Post-mortem examination and histopathological studies of affected organs, particularly in animals surviving for the observation period, may provide valuable information. Where different routes of exposure are used, e.g. oral, respiratory or parenteral, the relative hazard of different pathways of exposure can be assessed. The use of animals of both sexes as well as different species are common because there may be differences in acute toxic response.

Acute toxicity studies will identify highly toxic substances and information on the possible hazards of acute human exposure. In addition, the slope of the dose-effect and dose-response curves and the type of toxic responses observed are important for the design of subchronic, reproductive and toxicokinetic tests.

SUBCHRONIC TOXICITY

Subchronic tests are designed to assess toxic effects following regular daily exposure over relatively short periods of time (ranging up to 90 days). For many new chemicals, 28-30 days tests are becoming common because they can be used for notification purposes. Subchronic testing is important because it is the first, and for some chemicals, the only, repeated dose study. This means that every effort must be made to derive the maximum amount of information. A subchronic test should establish a spectrum of toxicological effects, their nature, target organs, severity and time course. Examination for delayed effects and determining whether or not they are due to the accumulation of a chemical is an important part of a subchronic study. It is important to establish if the toxic effects are reversible and a post-dosing observation period may be needed. A subchronic test may also indicate if particular toxic effects, for example, neurotoxicity, need further special testing.

A single species can provide an indication of potential for human health hazard but a demonstration of similar toxic effect and dose-effect relationships in two or more species greatly increases the relevance of the results to man. The dose-effect relationship derived from subchronic toxicity studies is used for setting doses for long-term carcinogenicity tests and can provide a usable no-observed-adverse-effect level. A subchronic test may, in the absence of chronic data, be used with caution for establishing acceptable daily intakes (ADIs) for substances such as food additives, or for setting threshold limit values or maximum acceptable concentrations for workplace exposure.

CHRONIC TOXICITY - CARCINOGENICITY

Although subchronic toxicity testing with comprehensive histopathology, and complemented by toxicokinetic studies, can provide valuable information about the toxic effects of a chemical, it has limitations. For example, subchronic tests are not reliable for the prediction of carcinogenic or mutagenic effects and are not designed to investigate teratogenesis. They cannot detect other effects on reproduction except for direct effects on the gonads. Thus, a full toxicological testing program requires chronic toxicity testing, involving the exposure of animals for a major part of their lifespan to examine the effects of a chemical on organs and tissues. Similarly, the objective of a

carcinogenicity test is to determine if long-term exposure to a chemical causes neoplastic lesions. Comprehensive and careful histopathology is a crucial element in the interpretation of these tests. Chronic and carcinogenicity testing require extensive laboratory facilities, careful planning, a reliable source of identifiable test animals, and excellent animal husbandry because intercurrent disease or early death of animals can ruin a test. The dose-effect and dose-response relationships obtained in chronic test should provide reliable no-adverse-effect-levels and define thresholds for chronic toxic effects. These data are used extensively for setting human exposure limits.

Incorporating Qualitative Information

The importance of the qualitative decisions is evidenced in the process by which both governmental agencies and industry determine how to handle a potential carcinogen. It is initially a two-step procedure in which a qualitative decision is made as to whether the compound is an animal and/or potential human carcinogen. If the answer to either of these questions is yes, then the risks to humans are estimated and a decision is made as to how to handle the compound. If a complete risk estimation model existed, there would be no need for the first step. The model would be used to compute risks directly and noncarcinogens would be assigned very small or zero risks. Unfortunately, our current state of knowledge does not permit us to take this approach.

Type of Tumor is Important for Risk Assessment

There are many specific qualitative decisions points that impact the quantitative estimation of potentical risk. These qualitative data include the type of tumor observed in the experimental animal. The B6C3F1 mouse, for example, is highly sensitive to hepatic tumors and the majority of male Fischer 344 rats develop testicular tumors independent of any chemical exposure. Chemicals that increase the incidence of these tumors but do not show any other tumorigenic activity should be considered as possible promoters of tumorigenesis. Such chemical are considered to be much less likely to cause human cancer than a potential carcinogen that produces a spectrum of histogenically different tumors including those with low spontaneous rates. The mechanism of action for some promoters is entirely different from that of complete carcinogens and certain steps in the assessment of risk should reflect the different mechanisms.

Examples of chemicals that only increase the spontaneous liver tumor rate for B6C3F1 mice after chronic high exposures, and are apparently not carcinogenic in rats, and do not appear to be mutagenic or genotoxic, include chlorinated solvents, perchloroethylene, and trichloroethylene. These substances do not appear to present a significant carcinogenic risk to humans at present exposure levels.

Tumor Type is also Relevant for Risk Assessment

A similar type of qualitative information that impacts estimates of potential risk is the relevance of the observed tumors in animals to potential human hazard. Examples of tumors that have limited predictability to humans from the animal model include zymbal gland carcinomas and tumors of the nasal turbinate. An excellent example is formaldehyde which produces tumors apparently only in conjunction with chronic irritacion of the nasal passage-ways. The rat, as an obligatory nasal breather, is sensitive to chronic irritation of the nasal mucosa and develop tumors in this area after chronic exposure to a number of chemicals. While it is possible, of course, that excess human exposure would result in respiratoy tumors, model used to estimate potential risk must be capable of appropriately incorporating this qualitative observation. Mathematical models currently in use do not have this capacity.

Genotoxic and Nongenotoxic Mechanisms

Probably the most important qualitative difference between animal carcinogens is the distinction between carcinogens that operate primarily through direct genotoxic mechanisms and those that produce tumors through mechanisms other than direct interaction with DNA (i.e. epigenetic, nongenotoxic, or nongenetic mechanisms). The subject of tumorigenic mechanisms has received a great deal of discussion (WHO/IPCS, 1985, 1985a, 1987, 1990, 1990a). Briefly stated, some chemicals that are carcinogenic in animals also interact directly with DNA as indicated by results of short-term *in vitro* tests, or by direct measurement *in vivo* DNA alkylation and repair rates. Other chemicals that are tumorigenic in animals show virtually no activity in the genotoxicity tests and show no propensity to bind or interact with DNA. Research on some of these chemicals (e.g., saccharin, chloroform, trichloroethylene, and perchloroethylene) has shown that there is litle to no direct interaction with DNA. On the other hand, *in*

vivo tests indicate a dose-dependent acceleration in the rate of DNA synthesis at doses that correlate well with tumorigenicity and demonstrates an apparent tumorigenic mechanism and threshold.

In some of these case the likely mechanism of action is cytotoxicity resulting in cellular regeneration accompanied by an increase rate of DNA synthesis. DNA is constantly undergoing a low background rate of damage and subsequent repair. If this background rate is increased (e.g. when cellular damage necessitates an increase in the rate of DNA synthesis), the increase demand on the repair surveillance systems may lead to an increase probability of faulty DNA repair or the possibility of replication before repair is completed. This phenomenon can be demonstrated by the production of skin tumors following burns and the repeated freezing of skin with dry ice and liver tumors following partial hepatectomy.

Cytotoxicity and direct cellular damage leading to tumorigenesis is only one nongenotoxic mechanism for carcinogenesis. Other nongenotoxic mechanisms and examples include solid state carcinogens (polymers, asbestos), hormonal imbalance carcinogens (estradiol, DES), immunosuppressors (azothioprine), and promoters (phorbol esters, saccharin).

Mechanistic Information: Its Use in Risk Assessment

The subject of tumorigenic mechanisms is extremely complex. For example, when classifying chemicals with respect to carcinogenic mechanisms, we should keep in mind that genotoxicity is a continuous spectrum rather than a dichotomous classification. Some chemicals clearly have a direct genotoxic component while others are at or near the nongenotoxic end of the scale. When doing risk assessment, it is important to identify those compounds that apparently operate through mechanisms other than direct genotoxicity. The importance of the distinction between genotoxic and nongenotoxic mechanisms is that, according to current theory, all that is needed for directly genotoxic chemicals to initiate a tumor is a single molecular event; thus, threshold may not exist. We note, however, that the existence of protective mechanisms, such as DNA repair systems that are saturable, would imply the existence of a practical threshold.

On the other hand, for nongenotoxic mechanisms, more that a single molecular interaction is necessary to produce a tumor. The consensus of scientific opinion is that, for some of these mechanisms, as with other

toxicological end-points, either an absolute threshold exists or the dose-response curve is so flat as to be indistinguishable from zero slope at low doses (i.e., a practical threshold exists). In some cases, the existence of an observable precursor related to tumorigenicity (i.e., cellular toxicity, necrosis, and hyperplasia), or a change in the rate of DNA synthesis may provide a marker variable that can be used to predict the threshold below which the nongenotoxic mechanism ceases to pose a risk.

For instance, many chemicals increase the spontaneous rate of hepatic tumors in B6C3F1 mice. High doses of many of these compounds result in tumorigenicity that is often preceded in dose and in time by microscopically observable histological alterations. For these chemicals the experimentally observable tumor frequency data are often augmented by earlier quantitative signs of clinical and subclinical liver toxicity that can be most useful in establishing better measures of no-observed-effect-levels. In those cases, a practical threshold exists as well as a mechanism for observing and quantifying the threshold.

These chemicals should be viewed as promoters of tumorigenesis in animals and should be evaluated as such in the traditional manner or other quantitative toxicological phenomena with demonstrated no-effect levels. Knowledge of species differences in metabolism and all the accompanying scientific judgments should be use in estimating the corresponding safe level for humans.

Extrapolation of Quantal Data in Risk Assessment

Another major limitation of the models is the large statistical uncertainty in extrapolating data orders of magnitude below the observable range, particularly for quantal responses. Two sources of variation that contribute to the uncertainty inherent in tumor frequencies are statistical (binomial) variability which implies a large degree of variation in tumor counts and experiment-to-experiment variability.

Tumor Identification is Based on Subjective Judgment

It should be also kept in mind that the variation in tumor frequency is a function of the uncertainty involved in classifying lesions into categories of "tumors" and "non-tumors"; such as the subjective judgments used in

separating high grade hyperplasia of the liver from low grade carcinomas. It was emphasized in the various examination of data in the ED_{01} Study conducted by the National Center for Toxicologial Research that the distinction between carcinomas and "non-tumors" such as hyperplasia is a subjective judgment and may not be consistent from pathologist to pathologist (Squire, 1981). It is also important to recognize that this judgment is often most difficult to make at low doses because a dose-response may exist in the severity of lesion as well as the frequency of lesions. At high doses malignancy is often clearly defined; however, at low doses the responses are often equivocal and may be classified as tumors or nontumors, depending on the opinion of the pathologist.

Determination of the Dose to the Target Tissue is Critical

Another reason for the large uncertainty and inconsistency noted in the extrapolation process is the measurement of dose or exposure. The dose used in the modeling should be that which is seen by the target tissue rather than the nominal dose administered to the animal. This is especially important if the exposure route in the experiment is different from that of the human. If the nominal and effective doses are strictly proportional, the nominal dose is an appropriate surrogate. Often, however, the high doses used in a bioassay saturate normal detoxification and excretory mechanisms resulting in nonlinear relationship between nominal and effective doses. In this case, Michaelis-Menten kinetics are observed and may be accompanied by alternative metabolic and excretory pathways that can result in enhanced toxicity of the chemical. The overall consequences of nonlinear toxicokinetics is that toxicity may increase disproportionately with increasing dose. Thus, extrapolation from high doses at which detoxification is overwhelmed to low nonsaturated doses using models that do not account for nonlinear kinetics can greatly overestimate the potential risk.

The issue becomes even more complicated when one relates the animal studies to humans and tries to determine the appropriate target tissue in humans and the dose of the chemical seen by those tissues. Metabolic and toxicokinetic data can help answer these questions, but unfortunately, almost all dose-response modeling reported to date is based on doses administered to the animal with no regard for kinetic data.

LOCAL EFFECTS

Tests for local effects on the skin and eye are of particular relevance to human risk assessment. For example, chemicals in cosmetics are deliberately applied and accident exposure to chemicals, such as in the workplace industry, is a frequent occurrence. Standard test procedures have been developed for examining the effects of chemicals on the skin and eye. Common effects observed after application of a substance to the skin or eye of a test animal are reversible inflammatory responses or even corrosion which results in necrosis of tissue. The degrees of erythema, associated oedema and skin and eye damage can be scored numerically but, in all cases, a careful and systematic description of the procedures and effects is also need. Extrapolation is less easy, because the test species, (usually rabbit for skin and eye, occasionally guinea pig for skin tests) differ from humans in their response to irritant chemicals. Because these species tend to be more sensitive, the tests may exaggerate the risks, but this introduces a greater safety factor.

ALLERGIC SENSITIZACION

This can affect the skin, respiratoy and gastrointestinal tracts. Skin sensitization to chemicals is a widespread problem in the workplace and among the general population. Sensitization reactions of the respiratory tract can be caused by many natural substances such as pollen, hair and insect scales as well as by industrial chemicals. Gastrointestinal tract intolerance is assuming more importance because of food allergies. With the exception of skin sensitization testing in guinea pigs, which is well established, there is a lack of satisfactory animal models for studying allergic reactions of the respiratory and gastrointestinal tracts.

For skin sensitization testing most methods use guinea pigs whose immune system has been pre-stimulated by adjuvant treatment. These are treated intradermally or epicutaneously, with the test substance and then challenged a week or so later with another dose. A positve response is indicated by the induction of skin erythema and oedema by the challenge dose. There are a number of test methods but, in practice, the results are not directly comparable. The widely used guinea pig maximization test probably overestimates the sensitization risk for humans but, again, this introduces a greater safety factor.

REPRODUCTIVE TOXICITY

Reproductive toxicology covers the effects of chemicals on the entire reproductive cycle, from mating through pregnancy to sexual maturity of the offspring. It ranges from overt teratogenic effects to the more subtle influences of chemicals on the whole reproductive cycle. The potential of chemicals to affect the reproduction process has led to reproductive toxicity testing being required in many countries for drugs, food additives, pesticides and other chemicals. The increasing number of women in the child-bearing period of life employed in industry and also exposed to chemicals in the home has added to the need for this type of testing.

The reproductive system and function are complex. Testing must examine function in the male and female, mating behavior, the oestrous cycle, male and female fertility, implantation, pregnancy rate, embryonic and fetal growth and development, litter size, nursing behavior and lactation, viability, neonatal growth and development and sexual maturation. Reproduction toxicity involves a series of tests examining gametogenesis, embryonic development, fetal growth and post-natal development.

The design and dose patterns vary with to the part of the cycle being investigated. For example, treated males are mated with untreated females, or vice versa, or both sexes are treated prior to mating. In males, a full cycle of spermatogenesis should be covered. Growth and development of the embryo are extremely important. If *in utero* exposure to a chemical alters the structure and function of offspring it is a teratogen. If a chemical kills the developing embryo or causes a reduced rate of fetal growth without any detectable structural or functional alterations, it is embryotoxic or fetotoxic (depending upon the stage of development affected). To test for teratogenic effects, a chemical is given to the pregnant test animals (usually mice, rats or rabbits) at high doses during the period of organogenesis. For embryotoxic effects, a chemical is given throughout pregnancy at lower doses. In both cases, the contents of the *uteri* are studied. Other effects of chemicals on the reproductive cycle may require studies extending over several generations.

MUTAGENICITY

Heritable mutations in germ cells can result in defects in offspring. Mutagenicity is accepted as a potential hazard for humans. Although many

countries require some mutagenicity testing it is directed more to screening chemicals for potential carcinogenicity.

In mutagenicity testing, effects on the gene and chromosome are examined. A widely used test is the detection of point mutations in bacteria using special strains of *Salmonella typhimurium* or *Escherichia coli* with or without of a metabolic activation system based on liver S9 homogenate.

Mammalian cells grown *in vitro* are used to examine the ability of a chemical to damage chromosomes. Human cells such as lymphocytes and cells obtained from laboratory animals can be used. The induction of gene mutation in cultured mammalian cells *in vitro* can be detected by alterations at the gene loci responsible for the activity of the enzymes hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and thymidine Kinase (TK).

Another gene mutation test in the induction recessive lethal mutations in *Drosophila melanogaster.*

The mutagenic potential of a chemical and its metabolites can also be examined by *in vivo* mutagenicity tests in mammals in the intact animal. Tests include the detection of chromosomal damage by metaphase analysis of bone marrow cells, the micronucleus test, tests for germ cell damage such as the dominant lethal test, and effects on skin pigmentation (mouse spot coat test).

While basic mutagenicity screening procedures will detect the majority of potential chemical mutagens, the extrapolation of the results for human health risk assessment is difficult, particularly in any quantitative sense.

TOXICOKINETIC STUDIES

The routine use of toxicokinetic studies is not yet general because of the technical complexities. However, this is an important area because chemical toxicity is frequently due to metabolites. In extrapolating from test species to humans, similarities or differences in metabolic handling of a chemical are important in the risk assessment. For example, it is useful to be able to compare the kinetics of oxidative metabolism, and have quantitative information on the pathways and rates of formation of reactive intermediates.

Metabolic processes and pathways vary in different animal species. The activities of various enzymes such as mixed function oxidases, epoxide

hydrolases, glutathione-S-transferases, and glucoronyl transferases, vary within and between animal species. The tissue concentrations of detoxifying substances such as glutathione, have important effects on toxicity, and these substances also vary with species, nutrition, dose and kind of chemical, and concurrent exposure to other environmental chemicals. There are species differences in receptors for hormones and other regulatory molecules and a comparison of tissue receptor concentrations between animal species and humans may contribute to quantitative risk assessment.

With continuous exposure to toxic environmental chemicals longer lived animal species must have a more effective protective system against chemical toxicity. The longer lived species are, in general, larger and have a lower tissue oxygen tensions than the smaller, short-lived species. Oxidative metabolism, which is frequently responsible for the activation of chemicals to toxic and reactive intermediate, is heavily dependent on tissue oxygen concentration. Smaller animal species (mouse, rat, hamster) generally metabolize toxic chemicals more rapidly than larger species (dog, primate, human).

Dose is important, particularly when there is continuous high exposure. Under these conditions protective mechanisms and enzyme reactions may be depleted or inactivated, while, on the other hand, activating metabolism can increase. Comparison of dose effect and toxicokinetics in animal species and humans may be crucial for interpreting animal toxicity data and the assessing of human risk.

EPIDEMIOLOGY

Epidemiology can complement laboratory data when the exposure of a human population to a chemicals is known.

Epidemiological investigations may be descriptive or analytical. Descriptive studies deal with patterns of distribution of a desease, symptoms, physiological variable or any definable health condition in one or more human groups. Analytical studies test hypotheses about the aetiology of disease and may be cross-sectional, retrospective (case control) or prospective (follow-up) studies. Disease patterns are described on the basis of the occurrence. The two main measures are prevalence and incidence. Prevalence specifies how many cases of a disease are present at a given moment in a

population whereas incidence is a measure of the number of new cases occurring during a defined time interval.

To link a chemical cause with a human health effect the nature and extent of exposure must be known. Because human exposure to chemicals is always complex this is often difficult. Even where a specific chemical is being investigated, obtaining reliable information on exposure and exposure concentrations may present problems. Exposure may be measured or estimated by means of biological monitoring which gives information on the body burden, air samples from individuals breathing zones, spot samples in a workplace, classification of exposure by work area, type of work, occupation, or simply classified as either "exposed" or "not exposed". Epidemiological methods can assist in the determination of causes to the quantification of exposure-response relationships. In all cases, reliable information on exposure, morbidity and mortality is essential for good epidemiology.

Data screening may be used to look for cause-effect associations, and to generate of hypotheses. For example, registers of mortality or morbidity and registers of exposures may be linked.

In epidemiology demonstration of a chemical cause of a disease is difficult and it is important to choose a statistical strategy designed to minimize or avoid false positives and false negatives and to eliminate the effects of confounding factors. When establishing a cause and effect relationship, quantification of exposure-response, the assessment of possible synergistic or antagonistic effects with other chemicals and external or host factors such as age and sex, must be included.

EXTRAPOLATION IN RISK ASSESSMENT

Extrapolation is basically a mathematical process of estimating in values and terms of a series on either side of know values. Extrapolations are thus, sctrictly speaking, quantitative but in biology they may be semi-quantitative or even qualitative. Extrapolations may be from laboratory animals to humans, from laboratory species to environmental biota, from high to low exposure situations, from short-term to chronic exposure, from single to multiple chemical exposure, or from one chemical to another. Most toxicological extrapolations contain a large element of uncertainty.

A widely used extrapolation is from animal data to man. Because this provides the basis for the majority of chemical risk assessments, it is a crucial part of methodology. The use of animal toxicity studies in extrapolation is based on the assumtion that they are relevant for predicting toxic effects in humans because of the anatomical and physiological similarities of mammal species and similar responses to many toxic chemicals. However, prediction of toxicity in humans from animal data is dependent on many factors, including laboratory animal species, test design and the procedures used for extrapolation. For acute toxicity, humans are generally considered to be more sensitive than experimental species, but there are cases where animal species are more sensitive than man. Marked species differences also occur in response to the chronic effects of chemicals. Species differences in sensitivity to toxic chemicals are mainly related to differences in biotransformation. Metabolic rate is an important determinant and small mammals, such as those used in laboratories, have a higher metabolic rate than humans. Sensitivity may also depend on biotransformation producing either more or less toxic products as well as on the rate of biotransformation. Also included in the extrapolation from relatively small numbers of genetically homogeneous laboratoy animals to highly heterogeneous human populations, including specially sensitive individuals.

Empirical approaches have been developed for the extrapolation of effects in one animal species to another and to humans. Species conversion factors have been derived. One conversion factor is based on the assumption that equally effective doses can be calculated per unit of body surface area which, in turn, is equal to body weight x 2/3. Another conversion, the "body weight rule", relates acute toxicity and body weight.

To deal with uncertainties it is customary to apply safety factors to animal data which are used to provide safe levels of exposure for humans. A safe level is usually based on an experimentally established "no effect level (NEL)" modified by a safety factor. Safety factors are themselves arbitrary and can be related to the type of toxic effect, its reversibility, the shape of dose-effect curve, the degree of difference between test species response, bioaccumulation, and the quality of toxicological data.

In setting a safety factor one approach assumes that the human is 10 times more sensitive than the most sensitive test species and that within human populations there is a tenfold range of individual sensitivities. Thus a

safety factor of 100 (10x10) may be used in setting acceptable exposure limits. However, depending on circumstances, a lower or a higher safety factor may be applied.

ECOTOXICOLOGY TESTING

This is an area that is still being developed. Relatively few countries have requirements for these data even though concerns for the environment are being strongly expressed. At the present time, ecotoxicological data are generated mainly for pesticides but these are not always adequate to permit the full environmental impact assessment. The behavior of a chemical in the environment is important because the environment is an important source of long-term human exposure and persistent chemicals can thus pose a threat to human health.

Most regulatory test requirements for chemicals include physical and chemical properties with some tests relevant to environmental behavior such as biodegradability in water and soil, abiotic degradation in air and biological oxygen demand and effects on some environmental biota including toxicity and bioaccumulation in fish. The physical and chemical properties of a chemical can be useful in predicting environmental behavior. Thus,

- melting point
- boiling point
- vapor pressure

indicate the physical state of a chemical under ambient conditions.

- Density
- viscosity
- solubility in water
- particle size
- partition coefficient (n-octanol/water)

indicate behavior in aquatic media and allow some prediction of environmental distribution and the parts of an ecosystem likely to be affected.

Tests that provide information for predicting environmental behavior and fate are:

- vapor pressure curve
- solubility in water
- adsorption/desorption
- volatility from aqueous solution
- complex formation in water
- density
- particle size distribution
- viscosity
- surface tension.

These help to predict the mobility of a substance in the environment and indicate its likely distribution between air, water and soil assuming that accumulation or degradation are insignificant.

Test data for degradation, hydrolysis, persistence and accumulation will refine predictions. Partition coefficient and fat solubility help in predicting the extent of absorption, distribution and storage in biota.

Effects on some non-mammalian biota can be tested in the laboratoy. Test exists for:

- Alga, growth inhibition
- Daphnia sp., toxicity and reproductive effects
- Fish (various species) acute and prolonged toxicity
- Avian (various species) toxicity and reproductive effects.
- Earthworm, acute toxicity
- Terrestrial plants, effects on growth
- Bacteria in activated sludge, effect on respiration.

APPROACHES TO ECOTOXICOLOGY RISK ASSESSMENT

The extrapolation of data from a few test species studied under laboratory conditions to the multitude of species in the natural environment contains many uncertainties. Ecotoxicity testing using fish and birds is fairly well developed and a range of test species is available. The use of various test

species of fish and birds can provide data which may be relavant to indigenous species. The use of different species can also identify the nature and the extent of possible species variation in toxic response. In ecotoxicological testing it is important that an indication of the range of effects in different organisms at different concentrations.

Ecotoxicological test results often vary because of the natural variation in test species used. Inbred test species can reduce the degree of variation but are much less representative of the real environment. This variability means that data can only indicate a probability of an adverse effect on a population and it is important to follow up predictions by effective environmental monitoring and observation.

MATHEMATICAL MODELS

The use of mathematical models for estimating human health risks due to chemicals is mainly confined to carcinogenicity, although some countries have developed mathematical approaches as part of "toxicometrics" to estimate risks for other toxicological end-points.

For carcinogenic risk, a number of mathematical models have been developed. These are the probit, logit, Weibull, one-hit, multi-hit and multi-stage models.

The first three are based on the hypothesis of individual tolerances in a population where the minimum tolerance is taken as zero. Stochastic models, namely the one-hit, multi-hit and multi-stage, are based on the hypothesis that a positive response is the result of the random occurrence of one or more biological events. The one-hit model is based on a response occurring when a target site is affected by a single biologically effective unit of dose. The multi-hit model is an extension of the one-hit model and assumes that more than one unit of dose is needed to produce a response. The multi-stage model is based on the hypothesis that an effect such as carcinogenesis depends on the occurrence of a number of different random biological events with the time rate for each event being in strict linear proportion to the dose.

These models provide estimated dose-response curves. The shape of the dose-response curves in the low dose region is important because it affects the estimates of risk associated with low levels of exposure which are those

of interest for public health. The one-hit model is linear at low dose levels. The logit, Weibull and multi-hit models will be linear at low doses only under certain conditions and usually the dose-response curves approach zero at a sub-linear rate. Similarly, the multi-stage model is linear at low doses only under certain conditions. The probit model is inherently sub-linear at low doses and generally gives to relatively low estimates of risk at low exposure levels. However, under other conditions the dose-response for the logit, Weibull and multi-stage models can approach zero at a supralinear rate indicating an increased risk. Low-dose linearity in the logit, Weibull and multi-hit models, is found with dose-response curves that are linear at low and moderate doses and decrease at high doses. Complementary metabolic and pharmacokinetics data are important in these extrapolations.

Since all these models are based on different assumptions they will give different results for the same data. Thus, in the use of mathematical approaches to toxicity and ecotoxicity, risk assessment for chemicals, scientific judgement remains paramount.

A brief detailed description of some of the most commonly used models are commented below.

Mechanistic Models

One class of mechanistic models is derived from assumptions about the age specific tumorigenicity rate which is defined as the proportion of people in any specific age group (e.g., 46-50 years) developing cancer. It is also often referred to as the hazard function. If the age specific rate (r) is a function of dose and age

$$r = f(\text{dose}, \text{age})$$

and if the dose and age components can be separated into two mathematical functions so that

$$f(\text{dose}, \text{age}) = g(\text{dose})\, h(\text{age}),$$

then the cumulative lifetime risk for a given dose is found by integrating over age. The resulting class of models is

$$P = 1 - \exp[-g(\text{dose})],$$

where P is the probability of tumor and g (dose) is a mathematical function of dose.

One-Hit Model

This family of distributions includes the one-hit model for which

$$g(dose) = b_1(dose)$$

and b_1 is a parameter to be estimated from data. This functional form assumes that for any given age, the probability of a tumor is directly proportional to the amount of exposure. This can result from the assumption that only one critical molecular event between a target site and the proximate carcinogen is sufficient to result in a tumor, and the probability of such an interaction is directly proportional to the nominal concentration of the carcinogen. This mechanism is, however, not the only one that is consistent with this equation.

The one-hit model and variations on it utilizing upper statistical limits represents a highly conservative approach to the extrapolation problem.

The one-hit models assumes a dose-response that is approximately linear at low doses and concave downward over the entire dose range. The model, as commonly used, ignores the toxicological reality of non-linear dose-response mechanisms, saturation kinetics, no-effect levels or thresholds of a real practical nature. The one-hit model is, however, relatively insensitive to minor fluctuations in the data. It should be noted that linear extrapolation from the lowest observed effect level to a potential risk of 10^{-6} (one in a million) generally results in a safety factor of approximately 100,000. Assuming an observed response level of 10% which is about the lowest detectable effect level in a standard biossay, the safety factor = response/ desired potential risk = $0.10/10^{-6}$= 100,000. This indicates that the dose corresponding to a potential risk of 10^{-6} is approximately 1/100,000 of the lowest practically observable effect level.

The dose-response curve calculated from the one-hit model is essentially independent of the shape of the observed dose-response curve, gives very little weight to no-observed-effect levels (NOELs), and may not be predictive of potential risk at low levels. When good epidemiological data are available for comparison, it has in some cases been found that the one-hit model is not compatible with the human experience.

With appropriate species conversion, the one-hit model does, however, estimate an upper limit on the potential risk and may be useful in situations where an upper bound is of interest. For example, if the potential risk

calculated by the one-hit model is not acceptable, then there would be less need to consider other models. On the other hand, if permissible exposures predicted by the one-hit model are unrealistically low, which is often the case, then further risk analyses would have to be made to confirm or refute the one-hit model results. In all cases it should be kept in mind that potential risks predicted by the one-hit model may be several orders of magnitude more than that of the true potential risk (factor of 10 = one order of magnitude).

Multi-stage Model

The multi-stage model assumes

$$g(dose) = (a_1 + b_1\, dose)(a_2 + b_2\, dose)\ldots(a_n + b_n\, dose)$$
$$= c_o + c_1\, dose + c_2\, dose^2 + \ldots + c_n\, dose^n$$

where a_1, b_1, $c_1 \geq 0$, are parameters that vary from chemical to chemical. The biological justification for this model is that cancer is believed to be a multistage process that can be approximated by a series of multiplicative linear functions $(a_1 + b_1\, dose)$. This model is likely to be *conceptually* useful in some cases. In a theoretical sense, for instance, the concentration of the proximate carcinogen at the target site can be modeled by a series of kinetic reactions that are usually assumed to be linear at low doses but may be saturable (nonlinear) at high doses. Concentration kinetics may be linear at low doses. This does not necessarily imply a proportional response because at some concentration the existence of defense and repair systems is likely to modulate the response.

In practice, however, this results in fitting a polynomial model to the dose-response curve. The function generally fits well in the experimental dose range but has very limited applicability to the estimation of potential risk at low doses. The limitations arise first because the model cannot reflect changes in kinetics, metabolism, and mechanisms at low doses; and second because low dose estimates are highly sensitive to a change of even a few observed tumors at the lowest experimental dose.

A logical statistical approach to account for the random variation in tumor frequencies is to express the results in terms of best estimates and measures of uncertainty. The following model provides an upper confidence limit on the potential risk but does not give a best estimate.

"Linearized" Multistage Model

This models is utilized by many of the regulatory agencies as a replacement for the linear term (b_1) of the polynomial function, g(dose), by its upper 95% confidence limit to reflect biological variability in the observed tumor frequencies. The dose-response predicted by this model is approximately linear at low doses (the d^2, d^3, etc., terms are essentially zero at low doses) resulting in estimates of potential risk that are almost identical to those of the one-hit model. Even for extremely nonlinear data estimated doses corresponding to potential risk levels of 10^{-6} differ only by less than a factor of 6 from the estimate of a one-hit model extrapolation. Thus, for almost all applications there is no appreciable difference between the linear model and the linearized multistage model.

This modeling approach relies totally on the upper confidence level for b_1 and ignores the best estimate of b_1 as well as the lower confidence limit. The result is that the model can produce a very high estimate of potential risk even when the dose-response function is very steep and when the exposure levels are well below no-observed-effect-levels.

The model has the potential to be applied even when the total dose-response is not statistically significant. This can be, however, very misleading.

Multi-Hit Models

One derivation of this model follows from the assumption that K "hits" or molelular interactions are necessary to induce formation of a tumor and the distribution of these molecular events over time follows a Poisson process. In practice the model appears to fit some data sets reasonably well and to give low-dose predictions that are similar to the other models. There are cases, however, in which the predicted values are inconsistent with the predictions of the other models by many orders of magnitudes.

Tolerance Distribution Models

Another approach to the modeling problem is to assume that each member of the population will develop a tumor if exposure to the carcinogen exceeds a critical level. This threshold level varies from individual to individual and has been modeled by various tolerance distribution.

Log-Probit Model

The log-probit model assumes that the individual tolerances follow a lognormal distribution. Specific steps in the complex chain of events that lead to carcinogenesis are likely to have lognormal distributions. For example, it is reasonable to assume that the distribution of a population of kinetic rate constans for detoxification, metabolism, elimination, as well as the distribution of immuno-suppression surveillance capacity of DNA repair capacity can be adequately approximated by normal or lognormal distributions.

Tolerance distribution models have been found to adequately model many types of biological dose-response data, but it is an overly simplistic expectation to represent the entire carcinogenic process by one tolerance distribution. A tolerance distribution model may give a good description of the observed data but from a mechanistic point of view there is no reason to expect extrapolation to be valid. The probit model extrapolation has, however, fit well in some instances.

Logit and Weibull Models

Other tolerance distributions which have been used to model carcinogenicity dose-response data include the logit and Weibull models. The multi-hit model discussed earlier can also be viewed as a member of this class of models that uses the gamma function to model the tolerance distribution. For this reason it is often called the gamma-multi-hit model.

The log-probit, logit, Weibull, and gamma distributions all have potentially similar shapes between tumor frequencies of 2% to 98%; hence, it is not surprising that these models often give essentially identical fits to the observed data, but again, the models differ widely at low doses.

Other Useful Models: No-Effect-Level Model

The observation that many biological responses vary linearly with the logarithm of dose, and that practical threshold exist, can be represented by the following models:

$$\text{Response} = B_1 \qquad \text{if dose} < d^*$$

$$\text{Response} = B_1 + B_2 \log(\text{dose}/d^*) \qquad \text{if dose} \geq d^*$$

This model incorporates a parameter d* that represents a threshold below which no dose-response occurs. In this model B_1 is the constant response level at doses less than d* and B_2 is the slope of the logdose-response curve at doses $\geq$ d*. It has been empirically found that many quantitative toxicological end-points can be adequately described by the no-effect-level model. This model may, therefore, be useful for establishing thresholds for end-points related to the carcinogenic process in situations where information other than the simple presence or absence of a tumor is available. Both the model and predicted threshold are of value when carcinogenicity is a secondary event.

Toxicokinetic Models

Toxicokinetic models have often been used to predict the concentration of the parent compound and metabolites in the blood and at reactive sites, if identifiable. The addition of statistical distribution for the rate of parameters and a stochastic component representing the probabilistic nature of molecular events and selection processes may represent a useful conceptual framework for describing the tumorigenic mechanisms of many chemicals. Toxicokinetic data are presently useful only in specific part of the risk assessment process. A more complete understanding mechanisms of chemically induced carcinogenesis would allow a more complete utilization of toxicokinetic data. Toxicokinetic comparisons between animals and humans are presently most useful for making species conversions and understanding the qualitative and quantitative species differences. The modeling of blood concentrations and metabolite concentrations identifies the existence of saturated pathways and adds to the understanding of the mechanism of toxicity in many cases.

In the future as more is understood about the mechanism of carcinogenesis, formalized quantitative approaches incorporating toxicokinetic data will likely become more useful in risk assessment.

The models described above, with the exception of toxicokinetic approaches, are generally an oversimplification of a complex system and apply only to the chronic animal toxicity studies that are but one input to the risk assessment process. The models have little biological relevance, have been shown to provide poor extrapolation estimates and, with a few exceptions, have not been validated either in animals or with respect to the human experience. Many of these models can be used, however, to summarize the

dose-response curve within the range of observable responses and toxicokinetic models are of use for understanding and predicting specified parts of the overall process.

Two major problems with the use of formalized modeling approaches are: 1) the inability of models to incorporate much of the qualitative information that must be used to arrive at logical decisions, and 2) the statistical problems involved in extrapolating quantal data.

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